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Occurrence of Quinolone-Resistance Genes in Ciprofloxacin-Resistant *Salmonella Enterica Serotype Typhi* Isolated form Blood Sample of Patients with Typhoid Fever.

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Abstract

Salmonella is approved as a common foodborne pathogen, causing major health problems throughout the world particularly in low- and middle-income countries. Low-level fluoroquinolone resistance is conferred by both chromosomal and plasmid-encoded resistance**,** this research was carried out look into the occurrence rate of *qnr*A,*qnr*B and *qnr*S genes in *Salmonella enterica serotype Typhi* Cipr ofloxacin-resistant insulate from blood samples of patients with typhoid fever. Fifteen *Salmonella enterica serotype Typhi* isolated previously from patients with typhoid fever were included in this study. All bacterial isolates were confirmed to have ciprofloxacin resistant by VITEK 2 microbial identification system; after plasmid DNA extraction; multiplex-PCR was done with primer sequences intended to plasmid-mediated quinolone-resistance genes which is *qnr*A, *qnr*B, and *qnr*S. In this study; it was 21 *qur* genes amongst 15 isolates. The *qnr*S gene was the commonest (10/21, 47.6%) followed by *qnr*A (6/21, 28.5%), whereas only 4 isolates were positive for *qnr*B (5/21, 23.8%). Some isolates had more than one *qnr* genes. So, Ciprofloxacin-resistant *Salmonella typhi* can have more than one gene at the same time; and the most occurrence rate in regards to qnr gene in this study was *qnr*S compared to *qnr*A and *qnr*B

Keywords: *Salmonella enterica serotype Typhi* , Typhoid fever, Fluoroquinolone, Ciprofloxacin, qnr gene.

وجود الجينات المقاومة للكوينولون في بكتريا السالمونيلا المعوية المقاومة للسيبروفلوكساسين النمط المصلي تايفي المعزولة من عينات الدم للمرضى المصابين بالحمى التيفوئيدية

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الخلاصة

تعتبر بكتريا السالمونيلا أحد مسببات الأمراض الشائعة المنقولة عن طريق الأغذية ، والتي تسبب مشكلة صحية كبيرة في جميع أنحاء العالم وخاصة في البلدان المنخفضة والمتوسطة الدخل. تحدث مقاومة الفلوروكينولون في هذه البكتريا عن طريق المقاومة المشفرة بالكروموسومات والبلازميد ، أجريت هذه الدراسة للتحقق من معدل حدوث جينات qnrA و qnrB و qnrS في سالمونيلا التيفوئيد المقاومة للسيبروفلوكساسين المعزولة من عينات دم المرضى المصابين بحمى التيفوئيد. تم استخدام خمسة عشر عزلة من السالمونيلا التيفية التي تم عزلها سابقا من مرضى مصابين بحمى التيفوئيد. في هذه الدراسة تم التأكد من أن جميع العزلات البكتيرية لديها مقاومة للسيبروفلوكساسين بواسطة نظام التعرف الميكروبي VITEK 2. بعد استخراج الحمض النووي البلازميدي تم إجراء Multiplex-PCR باستخدام بادئات تم تصميمها من اجل ان تستهدف جينات مقاومة الكينولون بوساطة البلازميد بما في ذلك qnrA و qnrB و qnrS. في هذه الدراسة؛ كان هناك ٢١ جين qur من بين ١٥ عزلة. كان جين qnrS هو الأكثر شيوعًا (٢١/١٠ ، ٤٧,٦٪) يليه qnrA (٢١/٦ ، ٢٨,٥٪) ، بينما كانت ٤ عزلات فقط موجبة لـ qnrB (٢١/٥ ، ٢٣,٨٪). بعض العزلات لديها أكثر من جين qnr. لذلك يمكن أن تحتوي هذه البكتريا المقاومة للسيبروفلوكساسين على أكثر من جين واحد في نفس الوقت. وكان أعلى معدل حدوث لجين qnr في هذه الدراسة هو qnrS مقارنة بـ qnrA و qnr

الكلمات المفتاحية: السالمونيلا التيفية ، حمى التيفونيد ، الفلوروكينولون ، سيبروفلوكساسين

Introductions

Salmonella enterica serotypes is a Gram-negative, rod shape, flagellated and aerobic bacteria; it is posing a great danger to human health, most notably in low- and middle-income countries [(1)].

For a long time, the first medication for Salmonellosis infections included chloramphenicol and trimethoprim in addition to penicillin, but the increase in resistance to such treatments prompted most doctors and with the emergence of newer types of antibiotics to use new antibiotics particularly Fluroquinolone groups [(2)]. Fluoroquinolone un-responsiveness are primarily caused by two mechanisms: chromosomally mediated mutations in topoisomerase's quinolone resistance determining regions (QRDR) and quinolone resistance determining region mutations; resistance genes belong to qnr groups plasmids mediated play a role in Fluoroquinolone resistant [(3)].

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Low-level fluoroquinolone resistance is conferred by both chromosomal and plasmid-encoded fluoroquinolone resistance [4]. Determinants belong to qnr have been found in a numeral of enterobacterial species from different parts of the world, including America, Europe, and Asia [5].

So far, six variants (*qnr*A1 to *qnr*A6) have been discovered. Quinolones produced by other plasmids *qnr*B (*qnr*B1 to *qnr*B5) and *qnr*S (*qnr*S1 and *qnr*S2) resistance determinants outlined in enterobacterial species [6].

The current study aimed to investigate occurrence rate of *qnr*A, *qnr*B and *qnr*S genes in ciprofloxacin-resistant *Salmonella typhi (S. typhi)* insulate from blood samples of patients with typhoid fever.

Material and Methods

Bacterial strain

A retrospective study of archived isolates, including 15 *Salmonella enterica serotype Typhi* isolates which was previously recovered from blood samples of patients with typhoid fever were used in this study. Resistance to ciprofloxacin for all bacterial isolates that included in the current study was detected by using of VITEK 2 microbial identification system, the detection was carried out according to manufacturing company (bio-Merieux). The bacterial isolates were diagnosed and classified in medical microbiology department at the faculty of medicine, AL -Nahrain University

Plasmid DNA extraction Protocol

Salmonella enterica serotype Typhi was harvested by using Luria-Bertani broth media, after centrifugation (8,000) rpm for two minutes; the supernatant was discarded and the pellet was collected. Plasmid extraction of the *Salmonella enterica serotype Typhi* was performed as described by company instruction (Wizard® Plus Minipreps DNA Purification System, Promega)

Multiplex-PCR was carried out with primer sequences specific for plasmid-mediated quinolone-resistance genes (Table 1), which is *qnr*A, *qnr*B, and *qnr*S. The settings of the PCR as follows: after initial denaturation at 94°C for 7 min, the 35-cycle amplification profile consisting of 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min using a cleaver scientific thermal cycler (TC 32/80-UK). The last elongation was place at 72°C for 10 minutes. For 1.5 hours, using 2% agarose at 7 V/cm (Merck-Germany) PCR product was identified. Concomitantly, a molecular marker (1-kb DNA ladder; Bioneer) was used. After the gel was stained with ethidium bromide, DNA bands were seen and photographed under UV light.

Table 1. Primer nucleotide for identification of plasmid-mediated quinolone-resistance genes

***Qnr* genes**		**Nucleotide sequences (5' ⟶ 3')**	**Products bp**	**References**
qnrA	**F**	GATAAAGTTTTTCAGCAAGAGG	**593**	7
	R	ATCCAGATCGGCAAAGGTTA		
qnrB	**F**	GATCGTGAAAGCCAGAAAGG	**469**	8
	R	ACGATGCCTGGTAGTTGTCC		
qnrS	**F**	TGGAAACCTACAATCATACATATCG	**656**	9
	R	TTAGTCAGGATAAACAACAATACCC		

Results

Multiplex PCR was used to check for the presence of the plasmid-mediated quinolone resistance genes *qnr*A, *qnr*B, and *qnr*S in 15 isolate of *Salmonella typhi* that were resistant to Ciprofloxacin; since amplicons product by PCR with the expected amplification product size *qnrA* gene (593 bp), *qnrB* (469 bp) and *qnrS* (656 bp) respectively (Figure 1).

Over 15 *Salmonella typhi* isolate; 21 *qur* genes were detected; and the *qnr*S gene was the most common (10/21, 47.6%) followed by *qnr*A (6/21, 28.5%), whereas only 4 isolates were positive for *qnrB* (5/21, 23.8%). Some isolates had more than one *qnr* genes (Table 2).

Table 2. Distribution of qnr genes through *Salmonella enterica serotype Typhi* isolates Ciprofloxacin resistance.

Isolates	qnrS	qnrA	qnrB	Total genes
1	+	-	-	1
2	-	-	+	1
3	+	+	+	3
4	+	-	-	1
5	+	+	+	3
6	-	+	-	1
7	+	-	-	1
8	+	-	+	1
9	-	-	+	1
10	-	+	-	1
11	+	+	-	2
12	+	-	-	1
13	+	-	-	1
14	-	+	-	1
15	+	-	-	1

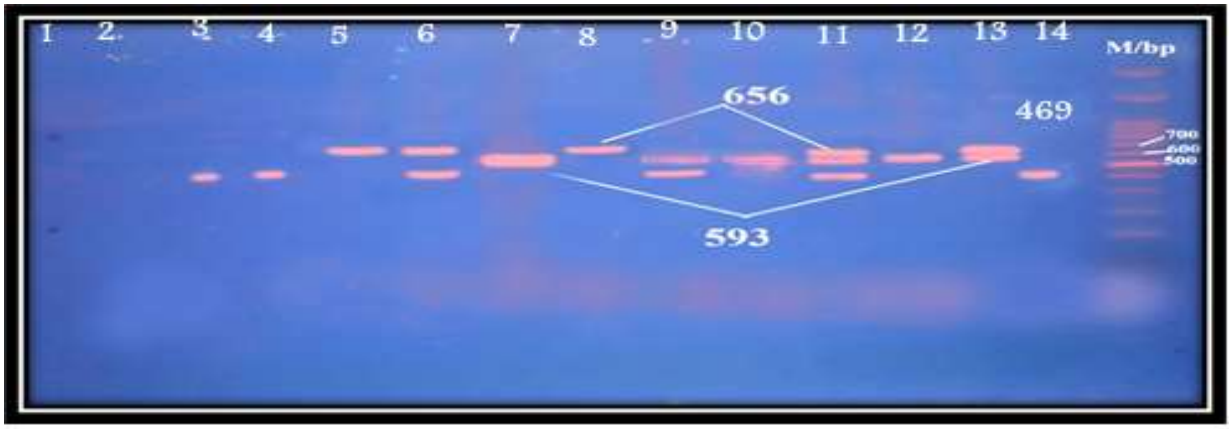

Figure 1. Gel electrophoresis of Multiplex PCR products (2% agarose, 7 v/cm^2,1.5hrs) for *qnr*A, *qnr*B, *qnr*S of *Salmonella enterica serotype Typhi* positive isolates. lanes 7, 9, 10,11, 12 and 13 *qnr*A gene (593 bp) positive isolates; lane 3,4,6,9,11,14: *qnr*B (469 bp) positive isolate; lanes 5,6,8,11,13 *qnr*S (656 bp) positive isolates.

Discussion

Previous studies have documented that *Salmonella* disease in humans can vary from self-limited gastroenteritis usually connected with non-typhoidal *Salmonella* (NTS) to typhoid fever with obstacles such as a fatal intestinal perforation; this bacterium has a propensity to acquire resistance to multiple classes of antimicrobial agents, and eradication of infection by highly resistant *Salmonella typhi* can be mostly difficult [(10)].

This study tested the occurrence of *qnr* genes among Ciprofloxacin resistance *Salmonella enterica serotype Typhi* isolated from the blood of patients with typhoid fever. Out of 15 *Salmonella enterica serotype Typhi* isolates;(21) *qnr* genes were detected. Two isolates harbored the three *qnr* genes *qnr*A, *qnr*B, *qnr*S whereas two isolates harbored two qnr genes in which one isolates harbored *qnr*A and qnrS while the second isolates have *qnr*A, *qnr*B.

Quinolone resistance is initiated mostly through chromosomal mutations. Quinolone resistance caused by plasmids has been reported in numerous places of the world in the last few years. This type of resistance is caused by plasmid-mediated *qnr*A, *qnr*B, or *qnr*S genes [(11)].

Quinolone resistance at low levels has been linked to DNA from transferrable plasmids. Several investigations have found that qnr determinants are widely distributed among bacterial isolates all over the world. Quinolones are antibacterial agents with a broad spectrum of action that are commonly utilized in both human and veterinary medicine. Their widespread use has been linked to an increase in quinolone resistance. [(11)].

Hopkins *et al*., mentioned that *qnr* genes contributed to high-level Ciprofloxacin resistance in Salmonella species in chromosomal and plasmid mediated [(12)].

There are many articles that reported an increase in non-susceptible bacterial strain to

fluroquinolone agents due to harboring the qnr genes may be contributed to using of such antimicrobials in food-producing animals [13,14].

Interestingly; in the current study; the *qnr*S gene was more prevalent (47.6%) than *qnr*A and *qnr*B which were (28.5%) and (23.8%) respectively. *qnr*S gene can increased selective pressure on the drugs and subsequently contributes to resistance even in the absence of mutations and its it is more easily transmitted from animals to humans [15].

The prevalence rate of qnr genes among Gram negative bacteria varies depending on sample type and locational area; Most studies reported that; the regional distribution of *qnr*A genes is known to be wide [15].

Many Enterobacteriaceae species have been found to have *qnr*A-like determinants, and six variants have been identified in *qnr*A and *qnr*B which is (*qnr*A1 to *qnr*A6) and (*qnr*B1 to *qnr*B6) while *qnr*S (*qnr*S1 and *qnr*S2), genes for plasmid mediated quinolone resistance (PMQR) have been found on many bacteria in addition to Enterobacteriaceae such as pseudomonas species with varying in size and incompatibility specificity [16].

Cameron-Veas *et al*. mentioned that 15% of *Salmonella enterica* in Brazil which have been isolated from pig harbored *qnrB*, and none was carrying *qnrA* and *qnrS* [17]. While in China Lin D *et al*. reported that (66%) Salmonella species carrying *qnrS* [18].

It's worth noting that the transfer of resistance genes among Enterobacteriaceae bacteria, such as quinolones genes, is a complicated process involving a variety of mechanisms, such as plasmid-mediated resistance gene transfer and chromosomal alterations. Types of clinical isolates, geographic location, and antibiotic usage rates in each country can all influence these pathways [19].

Many individuals in our country randomly use Ciprofloxacin without following clinicians' prescriptions in self-medication. In addition, the use of this type of antibiotic in the treatment of animals could result in greater selective pressure on such groups of antibiotics, which could lead to resistance through the different types of *qnr* genes.

Conclusions

This study reported that ciprofloxacin-resistant *Salmonella enterica serotype Typhi* may harbor more than one gene at the same time; and the most prevalent qnr gene in this study was *qnr*S compared to *qnr*A and *qnr*B. As far as we know, this is the first study in our country reported that results in *Salmonella enterica serotype Typhi* clinical isolates.

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